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Indiana Spring 2012 Investigation of Bee Kills in Relation to Alleged Pesticide Exposure and Planting of Treated Corn Seed

Guideline Requirements

US EPA OCSPP Guideline No. 850 SUPP

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Completion Date

October 10, 2012

Submitter:

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Company: Bayer CropScience

Company Agent: Clive Halder Ph.D.

Title: Director, Regulatory Affairs

Signature:

Date: October 10, 2012

The above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

Good Laboratory Practice Statement

This report does not meet the requirements for EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160, and differs in the following way:

(1) This report is not subject to Good Laboratory Practices.

Sponsor/Submitter: October 10, 2012

Clive A. Halder Ph.D. Date
Director, Regulatory Affairs

1.0 Background

On April 19, 2012, elevated bee mortality incidents that occurred in the north central portion of Indiana were reported to Mr. Mark O'Rourke of Bayer CropScience by Mr. Dave Shenefield of Clover Blossom Honey Farm (CBH). Mr. Shenefield operates approximately 2300 hives in nearly 100 apiaries and is the president of the Indiana State Beekeeper's Association. Other stakeholders in the farm include Mr. Shenefield's [redacted].

Dead bees were first noticed at the entrances of hives on April 13 in an apple orchard where 40 hives were placed for a period of 10 days to provide pollination services. This orchard is adjacent to corn fields that were planted during this time. This incident occurred near the end of apple bloom. Hives at the orchard were removed April 16 and placed at other apiaries including the airport and gravel pit apiaries.

An additional bee mortality incident occurred on April 19 at the workshop apiary. This apiary is a queen breeding apiary of CBH in which over 40 hives are located in addition to numerous nuc colonies. Similar to the events occurring at orchard apiary, dead bees were observed at hive entrances. A corn field was planted to the west and southwest of the workshop apiary on the previous day. This field had been recently sprayed with herbicide to kill the cover crop that had been established in the late summer and fall of 2011. According to the Shenefields, the wind was coming from the south and dust was noted drifting toward the apiary at the time of planting on April 18. The Shenefield's report that significant bee mortalities continued for a few days at the workshop apiary hives until returning to a normal level after April 22.

Dead bees observed at the airport apiary (left), 24 April; and workshop apiary (right), 25 April

On April 19, the Office of the Indiana State Chemist (OISC) was notified of the incident at workshop apiary. The following day, an OISC investigator visited the site and collected samples for analysis. The case summary for this incident is included in appendix A.

Mr. O'Rourke met with the Shenefields in Indiana on April 20 and conducted a preliminary tour of the area where the kills occurred. A more thorough investigation was conducted April 23-25 by Mr. O'Rourke and Mr. Dick Rogers of Bayer CropScience. At this time, the Shenefields indicated that higher than usual bee mortalities were also being observed at Shorty and cabin apiaries. On April 23-24, the corn field south of workshop apiary was planted after a failed burn down of abundant flowering bee-attractive plants. Numerous dead bees were observed at the entrances to and on the ground in front of these hives on April 25.

2.0 BCS Investigatory Actions

2.1 Field Methods

The field investigation team consisted of Mark O'Rourke and Dick Rogers of Bayer CropScience. The orchard and workshop apiaries were visited on April 23. "Shorty" apiary, located adjacent to a soybean field, was also visited on this day. On the following day, airport, workshop, and gravel pit apiaries were visited. On April 25 workshop and cabin apiaries were visited. After the bee incident observation at workshop apiary on this day, the equipment involved in the corn planting of the corn field south of the apiary was inspected.

Locations of apiaries, fields, driveways, and landmarks/significant features were marked using a 2004 version of a handheld Garmin 60CSx GPS. Longitude and latitude information were recorded for each location. Surrounding towns include La Fontaine, Marion, Wabash, and Mt. Etna, Indiana. Fields around affected sites were scouted with planting status and activity noted.

Dead bee samples were previously collected by Mr. Shenefield from the orchard apiary on April 13, when he first noticed elevated mortality. These were stored in a conventional freezer until shipment could be arranged to the analytical laboratory. Dead bee samples were collected by the field investigation team for pesticide residue analysis from all visited apiaries with an accumulation of dead bees. A sample of dead bees was also collected from the workshop apiary and live bee samples were collected from cabin and airport apiaries for pathogen evaluation.

Additional samples were collected from the corn field that was planted on April 24 immediately south of the workshop apiary on the following day. Samples of blooming bee-attractive plants were collected from this field along a rough transect to the apiary. Samples consisted of whole flowers, with nectar, pollen, and any dust associated from the planting not being assessed separately.

Bee attractive plants associated with the corn field planted just prior to the workshop apiary bee incident of April 25. The right picture shows the corn field with the workshop apiary in the background. The left picture shows plants between the corn field and the apiary.

On April 25, the equipment used to plant the corn field south of the workshop apiary was examined. This was a John Deere finger pick-up style planter. Seed was collected from two of the row seed boxes and the corresponding seed meter units. The contents of the seed meter, which included seed as well as residual dust, was emptied into a sample bag and sent to the BCS Seed Technology Center in Research Triangle Park, NC for analysis using a Heubach dust meter. Corn seed used for this planting was primarily treated with Poncho (clothianidin) although some Cruiser (thiamethoxam) was also utilized.

Locations of plant samples taken in relation to the planted corn field (outlined in blue) and the workshop apiary (indicated by the cartoon hive)

2.2 Laboratory Analysis

Dead bee and plant samples were sent frozen on dry ice to the Bayer CropScience Residue Analysis group in Stilwell, KS (hereafter referred to as BCS-Stilwell) where each group of bees was divided into two subsamples, with one subsample analyzed by BCS-Stillwell, and the other subsample sent to the USDA Agrimarketing Analytical Services Lab in Gastonia, NC (hereafter referred to as USDA-Gastonia). Subsamples consisted of different groups of whole bees (5 per subsample) collected from the same hive. At the BCS-Stilwell lab, subsamples were analyzed for the presence and quantity of clothianidin using a LC/MS/MS analytical method that has been used in previous studies reviewed by the US EPA. The USDA-Gastonia lab analyzed subsamples for the presence and quantity of approximately 200 different pesticide active ingredients and metabolites including clothianidin and thiamethoxam using LC/MS/MS and GC/MS/MS methods that have been used in several previously published studies (e.g., Mullin et al. 2010).

Assessment of *Varroa* mite infestation and *Nosema spp.* spore counts were conducted at the Cedar Grove Research Facility of Eurofins Agroscience Services (Mebane, NC).

3.0 Results

3.1 Interview with Mr. Dave Shenefield

In discussions with Mr. Shenefield, he indicated that he noticed bee losses that were potentially related to corn planting in 2010 at CBH apiaries located near Manchester, Indiana. However, these observations were limited to a few locations and CBH personnel were not actively looking for dead bees as the apiaries were worked. Weather in 2010 was very warm and windy in the spring season and corn planting started during the second week of April. No report was made to the Indiana state chemist regarding this potential bee kill; however, since this occurrence Mr. Shenefield has been looking for dead and dying bees associated with the spring corn planting season.

Mr. Shenefield did not observe unusual numbers of dead bees during the corn planting season of 2011. It was generally cooler and wetter than in 2010 during this time of year. Corn planting was delayed and many of the fields were not planted until June. Some fields were not planted in corn at all. The field directly south and east of the workshop apiary was not planted in field crops that year, but was planted in a cover crop as a soil conservation practice.

During the spring of 2012, CBH personnel observed dead bees in front of some portion of the hives at 30-50 percent of the operating apiaries. No hives were completely lost, however a brood cycle was reportedly lost in affected hives. To compensate for high worker bee mortality and loss of brood production, CBH added frames of healthy brood to the weakened colonies in an effort to bring them back to a level where they could contribute to honey production. Mr. Shenefield noted that it was the strongest hives, those with a large field force, that were affected the most. In the queen breeding operation, approximately 500 queen cells were lost during a time coincident with corn planting.

3.2 Results of Survey of Surrounding Landscape

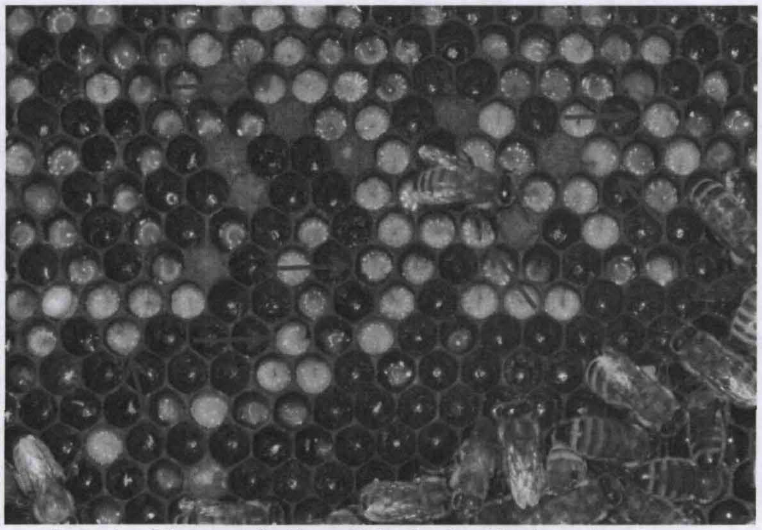
Timing of planting in 2012 in the area surrounding the apiaries was well ahead of the 5-yr average for corn and soybean planting in Indiana. Weather conditions during March and early April were generally much warmer and dryer than usual. Corn planting was winding down at the time of the field visit to CBH apiaries. The field the corn field south of workshop apiary that was planted on April 23-24 was the last of the corn fields planted on that farm for this season. Soybeans were being planted in the area during the field investigation team's visit (April 23-25).

Several of the fields surrounding the workshop apiary were planted to a cover crop in 2011 and a burn-down application of herbicide had been applied prior to planting. Fields located directly south and east of the workshop apiary as well as the field southwest and west had been planted following the cover crop desiccation. In both fields, there were areas of cover that had not been completely controlled by the herbicide and strips of crimson clover had survived and were present.

Map of workshop apiary and surrounding fields

3.3 Inspection of Hives

Two hives were inspected at the airport apiary which contained hives that were previously located at the orchard apiary. These hives contained evidence of starvation with some nectar coming in and queens present and laying. These hives did not receive supplemental syrup feeding so Mr. Shenefield was not surprised that these were showing some signs of starvation. European foul brood symptoms were observed in these hives.



Example of European foul brood infection in a hive from airport apiary

At workshop apiary, three hives were opened; however only the top boxes were assessed. This was due to cold weather conditions and to avoid the risk of damaging breeder queens by going into the brood nest.

The gravel pit apiary is a holding yard with hive numbers varying from 100 to 200. Eight hives previously located at Tuttle orchard were being held here. Six of these hives were opened. Five of these were doing well, while one was queenless. All colonies were being fed sugar syrup and no symptoms of starvation were observed.

The cabin apiary is set in a large woody area with only small planted fields in the vicinity. Approximately 40 hives were kept at this location with hives and bees noted as being in poor condition.

3.4 Pesticide Residue Analysis Results

Residues from dead bee samples are summarized in Table 1. Clothianidin residues were measured in all samples by both analytical laboratories. Across all locations, average clothianidin levels were 6.3 and 9.6 ppb as measured by BCS-Stilwell and USDA-Gastonia. A two-tailed paired t-test ($\alpha = 0.05$) did not reveal a significant difference in the measurements between the two laboratories. With the exception of those samples taken from the airport apiary, all of the samples possessed average residues of clothianidin above 5 ppb. These measurements are somewhat higher than the residues measured by the Office of Indiana State Chemist Residue Lab (see Appendix A).

Thiamethoxam was detected in three samples: one each from the orchard, airport, and workshop apiaries, respectively. It should be noted that clothianidin is a primary metabolite of thiamethoxam and as such the clothianidin residues measured in dead bees could be due to exposure to dust released from seeds treated with either of these insecticides, or from both.

A third neonicotinoid insecticide, acetamiprid, was also detected in dead bee samples from hives from the orchard, airport, and workshop apiaries. Residue levels of acetamiprid were particularly high in orchard apiary samples 002-1 and 002-2, with measured values of 897 and 1560 ppb. These samples differed from the 003-1 and 003-2 in that they were collected from different locations within the orchard. These samples were collected on April 23 which was after the hives had been moved from this apiary on April 16. Therefore they had been present on the ground for at least one week following the mortality event. Sample 005-100 was collected at the orchard location on April 13, the day when the mortality event was first noted at this apiary. This sample did not contain detectable levels of acetamiprid. This suggests that acetamiprid was applied after the mortality event occurred. The high residues measured on samples 002-1 and 002-2 (collected on April 23) are consistent with levels that would be expected if dead bees on the ground were oversprayed.

Thymol was detected in all samples and had the highest overall average residue level of the compounds included in the multi-residue method. This is a plant-derived monoterpenoid that is used in the control of *Varroa* mites where it is thought to act as a neurotoxicant (Johnson et al., 2010). Although this product has been used routinely in apiculture, some studies indicate it may have sub-lethal, adverse effects in honey bee hives (Johnson et al., 2010).

Clothianidin was detected in all plant samples associated with the corn field south of the workshop apiary by BCS-Stilwell (Table 2), although levels were reported by USDA-Gastonia as below the limit of detection at the two locations farthest outside this corn field. In general a gradient of clothianidin levels existed, with the highest levels being found in plants growing in the field and lower levels found as the sampling location moved toward the workshop apiary. The herbicide, atrazine was detected in all plant samples as well as in all dead bee samples. This pesticide however is considered virtually non-toxic to honey bees.

The total Heubach dust level and clothianidin dust level of the samples recovered from the planter boxes (hoppers) and meters are summarized in Table 3. These seeds had graphite planter lubricant applied so the majority of the dust was due to the graphite and not the dust from the treated seed. In the absence of the same lot of seed without the applied graphite, the true dust level released by treated seed cannot be determined.

3.5 Bee Parasite and Pathogen Results

Parasite and pathogen analysis results are detailed in Table 4. *Varroa* mite counts in samples of bees collected live from affected hives ranged from 0.4-3.4 mites per 100 bees. *Nosema* spore counts varied tremendously between the apiaries. Hives from the airport and workshop apiaries had spore counts below 5 million per bee, the level at which rescue treatments are typically prescribed. The hive from the cabin apiary however had a spore count of 168 million per bee indicating a very high level of infestation.

4.0 Discussion

Climatic conditions in the spring of 2012 in Indiana were unusual in that warm temperatures began earlier and conditions were dryer. The earlier spring resulted in earlier blooming of perennial crops and wild plants as well as an earlier start of honey bee brood production, which in turn led to faster growth of colonies. When this happens, there is always the risk that a return to cold weather could damage early blooming plants and adversely impact the availability of nectar and pollen, leading to starvation and nutritional deficiency in hives. These conditions will also exacerbate disease outbreaks and lead to increased competition between colonies.

There was evidence of starvation/nutritional deficiency observed in the inspected hives at the airport apiary. These hives were examined a week after the associated mortality event (which occurred when the hives were at the orchard prior to being moved to the airport) so it is not clear if the conditions observed here were causal or symptomatic of the initial event. These hives were not being supplemented with sugar syrup. Hives examined at the gravel pit apiary had also been recently moved there from the orchard. These hives were receiving supplemental food (sugar syrup) and were noted to be in better condition.

In this part of Indiana from April 1 to 25, only 2.5 cm of precipitation occurred. This minimal rain coupled with unseasonably warm temperatures led to dry fields containing bee-attractive blooming plants. Burn-down of these blooming plants with herbicides was poor in some cases, but corn planting proceeded anyway. These early, dry conditions, coupled with the presence of blooming bee-attractive plants in the fields appear to be ideal for generating dust that can settle on flowers in the fields and along field borders. In addition, the month of April in Indiana was windy with high winds ranging from 16-45 mph. Even very low amounts of dust could be carried significant distances off-site in such conditions.

Past investigations of bee mortality incidents believed to be caused by exposure to clothianidin-laden dust have generally found clothianidin residue levels to be greater than 5 ppb in dead bees sampled from affected hives (Pistorius et al. 2009). With only a few exceptions, dead bee samples collected in this investigation had average clothianidin levels (i.e., the average of the measurements made by the two analytical labs) above this threshold. This suggests that clothianidin very likely contributed to the observed mortality. Those samples with average clothianidin residues below 5 ppb were collected from the airport apiary from hives that were previously located at the orchard apiary, the site of onset of the observed bee mortality event. These samples were therefore collected after the hypothesized exposure, both temporally and spatially. The source of bee exposure to clothianidin appears to have been from dust released during planting of treated corn seeds; however, it is not known to what extent the source seed had been treated with clothianidin itself, or with thiamethoxam, a precursor molecule that insects metabolically transform to clothianidin. The presence of some samples with detectable levels of both molecules indicates at least some of the exposure was the result of the planting of thiamethoxam-treated seeds.

Relatively high levels of acetamiprid were measured from samples collected at the orchard apiary. This insecticide is significantly less toxic to honey bees than clothianidin, with the contact LD50 comparisons showing that acetamiprid is 324 times less toxic than clothianidin (Iwasa et al. 2004). Assuming this holds similarly for residue values in dead bees, then the measured acetamiprid values would be at levels that could contribute to mortality. However, the available evidence indicates that these residues are more likely the result of bees already dead on the ground being present when the orchard was sprayed with an acetamiprid-based product, and as such are incidental findings.

Clothianidin residues were measured both in samples collected from the planter of the corn field associated with the April 25 bee incident at workshop apiary and from the flowers of plants between this field and the apiary. For various reasons these measured values do not equate with the exposure that occurred in this instance. The plant samples were of the entire flower and not the matrices via which exposure would have occurred (i.e., nectar, pollen, and dust). The samples collected from the planter are from the entire contents of the seed box and meter and do not reflect the dust that would have originated from the seed during the planting process. These values do, however, provide evidence for an exposure pathway that may have led to the bee mortality incident. Specifically, dust released from the planting of the corn field likely settled on blooming plants adjacent to the workshop apiary.

Foraging honey bees visiting these flowers may have come into contact with the insecticide-laden dust resulting in mortality when the exposure exceeded a toxic level.

The affected colonies did not die out as a result of these incidents of pesticide exposure. In fact, in a follow-up visit by Rogers and O'Rourke to CBH in late August 2012, Mr. Shenefield indicated that no further incidents of elevated worker bee mortality had been observed and that his operation's honey production was far above normal in 2012.

5.0 Conclusion

Residue levels of clothianidin in most bee samples were at levels at which mortality due to exposure to either clothianidin or thiamethoxam would be expected. Results are most conclusive for the bee mortality that occurred at the workshop apiary. At this site planting of corn fields to the south/southwest of the apiary occurred one day prior to the both incidents that occurred at this site. Neonicotinoid insecticide exposure is therefore the most likely causal factor in the observed incidents of bee mortality. Limited evidence of starvation, nutritional deficiency, and *Nosema* infestation were observed however these stressors may have contributed to some of the observed mortality in those apiaries in which they were noted. This incidents of elevated mortality of worker bees resulted in an early season setback to spring build-up of the affected colonies. While undesirable, the incidents were of brief duration and colonies were able to recover with the assistance of the management actions taken.

6.0 Literature Cited

Iwasa T, Motoyama N, Ambrose JT, Roe RM. 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop Prot 23:371-378.

Johnson RM, Ellis MD, Mullin CA, Frazier M. 2010. Pesticides and honey bee toxicity – USA. Apidologie 41:312-331.

Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, Pettis J. 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS ONE 5(3):e9754. doi:10.1371/journal.pone.0009754.

Pistorius J, Bischoff G, Heimbach U, Stähler M. 2009. Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. Julius-Kühn-Archiv 423:118-126.

Table 1. Residues, in ppb, of clothianidin in dead bee samples collected at entrance and in front of hives as determined by BCS-Stilwell and those analytes where any sample had a detectable residue by USDA-Gastonia. LOD is 1 ppb for both labs and all samples. Analyzed samples consisted of ~0.5 g of bee with samples ranging from 3 to 9 bees except where noted. Apiary codes are O=orchard, A=airport, S=Shorty, W=workshop, and C=cabin. Codes for chemical classes are CNI=cloronicotinyl insecticides, OP=organophosphate insecticide, Met=metabolite, HT=hive treatment pesticide, Fung=fungicide, and Herb=herbicide.

Sample ID	Apiary	Sample Date	Acetamiprid (USDA)	Clothianidin (BCS)	Clothianidin (USDA)	Thiamethoxam (USDA)	Chlorpyrifos (USDA)	DDE p,p' (USDA)	Coumaphos (USDA)	Fluvalinate (USDA)	Thymol (USDA)	Captan (USDA)	Carbendazim (MBC) (USDA)	Cyprodinil (USDA)	Trifloxystrobin (USDA)	Atrazine (USDA)	Pendimethalin (USDA)
			CNI	**CNI**	**CNI**	**CNI**	**OP**	**Met**	**HT**	**HT**	**HT**	**Fung**	**Fung**	**Fung**	**Fung**	**Herb**	**Herb**
002-1	O	4/23	1560	8.4	8.9	nd	nd	11.4	6.9	nd	218	nd	nd	nd	nd	25.5	nd
002-2	O	4/23	897	2.9	14.5	nd	nd	5.2	nd	nd	147	64.9	nd	nd	nd	37.3	6.6
003-1	O	4/23	nd	10.8[1]	nd	nd	nd	nd	nd	nd	296	nd	nd	nd	nd	50.5	32.8
003-2	O	4/23	8.5	3.9	13.8	4.8	nd	nd	2.2	nd	425	nd	16.7	nd	nd	40.2	nd
004-1	A	4/24	7.2	0.5	7.8	3.9	nd	nd	nd	nd	188	nd	14.6	nd	nd	7.0	nd
004-2	A	4/24	5.9	2.9	2.4	nd	nd	nd	nd	nd	124	nd	10.5	nd	nd	12.9	nd
004-3	A	4/24	5.6	1.4	3.1	nd	nd	nd	1.8	nd	196	nd	9.0	nd	nd	6.4	nd
005-100[2]	O	4/13	nd	14.3	7.1	nd	nd	nd	1.8	nd	126	nd	nd	nd	nd	13.0	nd
005-101	S	4/20	nd	6.2	8.4	nd	nd	nd	nd	5.4	171	nd	nd	nd	nd	6.4	nd
011-1	W	4/25	nd	4.9	11.3	nd	nd	nd	nd	nd	148	nd	nd	nd	nd	20.1	nd
011-2	W	4/25	2.3	4.8	25.7	nd	nd	nd	nd	nd	135	nd	nd	33.2	nd	24.0	nd
011-3	W	4/25	nd	4.7	9.1	nd	nd	nd	nd	nd	60.5	nd	nd	13.6	nd	11.8	7.2
011-4	W	4/25	3.4	15.3	9.0	nd	nd	nd	nd	nd	201	nd	5.5	nd	nd	15.3	nd
011-5	W	4/25	2.5	7.0	10.3	5.5	2.3	nd	nd	6.1	72.4	nd	nd	nd	nd	33.6	nd
012-1	C	4/25	nd	11.5	3.1	nd	nd	nd	1.9	61.6	132	nd	nd	nd	nd	6.4	8.2
012-2	C	4/25	nd	5.1	8.8	nd	nd	nd	nd	23.1	119	nd	nd	nd	8.9	9.1	5.6

nd = not detectable

[1]This sample was highly desiccated and consisted of 25 bees. [2]This sample was collected by Mr. Dave Shenefield.

Table 2. Residues, in ppb, of pesticides in samples of blooming bee-attractive plants collected along a rough transect from center of a field planted with treated corn seed to the workshop apiary where bee mortalities occurred. Results determined by BCS-Stilwell and USDA-Gastonia Limit of Quantification (BCS & USDA) = 1 ppb.

Sample Number	Plant Description	Clothianidin (BCS)	Clothianidin (USDA)	Imidacloprid (USDA)	Thiamethoxam (USDA)	Atrazine (USDA)	Metolachlor (USDA)	Metribuzin (USDA)
015-1	Crimson Clover	0.2	nd	nd	nd	335	nd	nd
015-2	Crimson Clover	5.4	8.1	nd	3.7	208	nd	nd
015-3	Crimson Clover	6.3	8.0	nd	nd	399	nd	nd
015-4	Crimson Clover	3.5	3.9	nd	nd	166	nd	nd
015-5	Mustard	5.8	4.7	nd	nd	368	10.7	1.1
016-1	Mustard	3.6	3.4	nd	nd	168	nd	1.2
016-2	Yellow Rocket	3.5	3.9	nd	nd	88.1	nd	nd
017-1	Plant	0.5	nd	nd	nd	42.4	nd	nd
017-2	Mustard	0.7	nd	nd	nd	102	nd	nd
017-3	Mustard	0.5	nd	nd	nd	112	nd	nd
018-1	Mustard	1.1	nd	nd	nd	109	nd	nd

nd = not detectable

Table 3. Heubach dustmeter test results on samples from corn planter hopper box and meter. Samples included treated seed along with residual dust present in the planter.

Treatment & Planter Lubricant	Total Grams dust per 100,000 kernels	Grams clothianidin in total dust per 100,000 kernels (HPLC analysis of filters)	% clothianidin in dust
Seed Box Sample Average of 2 Row Units AgVenture 2012 Dawes, Indiana	1.68	0.08	4.8%
Seed Meter Sample Average of 2 Row Units AgVenture 2012 Dawes, Indiana	6.81	0.26	3.8%

Table 4. The presence of *Varroa* mites and *Nosema* spores were evaluated by Eurofins Agrosciences Services.

Sample Number	Apiary	Collection Date	*Varroa* Mite (#/100 bees)	*Nosema* spore (#/bee)
004-100	Airport	April 24	2.7	75,000
004-200	Airport	April 24	0.5	1,075,000
011-pooled*	Workshop	April 25	3.4	475,000
012-3	Cabin	April 25	0.4	167,820,000

*Dead bee samples

Personal privacy information

Appendix A. Case Summary from the Office of the Indiana State Chemist

CASE SUMMARY

Case #2012/0700

Complainant: Beth Shenefield
[redacted]

1. On April 19, 2012, Beth Shenefield contacted the Office of the Indiana State Chemist (OISC) to report that large quantities of her bees were dying/dead. Mrs. Shenefield suspected the bee deaths were in response to corn being planted in a neighboring field.

2. On April 20, 2012, I met with Shenefield at her hives/ place of business. Mrs. Shenefield stated that a neighboring farmer was planting corn in the field south of her residence on April 18. She said the wind was blowing from the farm field toward her hives. She then explained that at about noon on that same day, she observed what she considered odd behavior among the bees. She said the bees were having spasms and could not fly. Mrs. Shenefield was unable to estimate how many bees died, but she thought it to be in the hundreds or thousands.

3. Upon observing the hives, I saw multiple clumps of dead bees. I also noticed that some of the bees were disoriented and sluggish. Ms. Shenefield then collected a jar of dead bees. She also gave me a sample of bees that she had collected at an earlier time and put in the freezer. Both samples were submitted to the OISC Residue Lab.

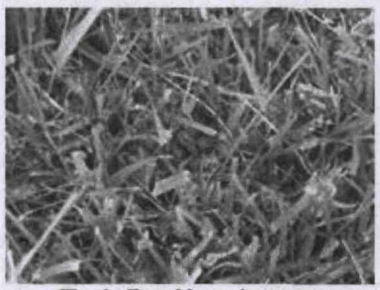


Fig 1: Dead bees in grass

Fig 2: Zoomed out photo dead bees

Fig 3: Farm field planted (approx quarter mile away)

4. The OISC Residue Lab reported finding 2.5 parts per billion of clothianidin in the sample collected during my investigation and 3.1 parts per billion of clothianidin in the sample collected by Mrs. Shenefield at an earlier time. Clothianidin is an active ingredient in the seed treatment of corn.

Elizabeth Carter
Elizabeth C. Carter
Pesticide Investigator

Date: June 11, 2012

Disposition: Although the pesticide clothianidin was detected in the bees, no violation of the Indiana Pesticide Use and Application Law could be documented. The information was forwarded to USEPA and to selected bee specialists and researchers for further review and possible determination of source of exposure to the bees.

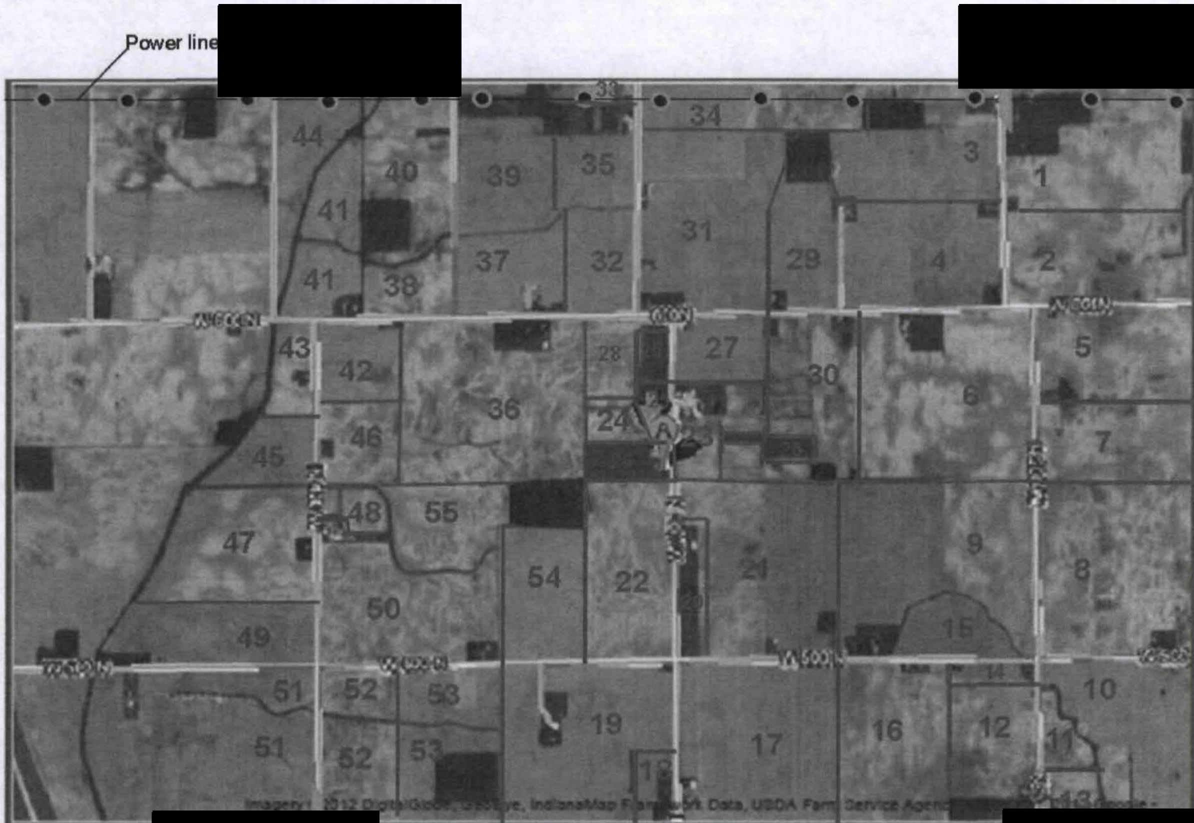
George N. Saxton
George N. Saxton
Compliance Officer

Final Date: June 11, 2012

Personal privacy information

Appendix B. Survey of Fields in the Vicinity of the Orchard Apiary

[redacted] [redacted]



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Field #	Crop	Crop Stage	Tillage	Corn Hybrid Brand	Planter Type	Weeds	Comments
1	Corn	V5	Min. till			Clean-trace weeds, STEME	TAROF, PLALA, TRFRE in ditch
2	Corn	V5	Min. till			Nearly clean, few STEME	TAROF, PLALA, TRFRE in ditch
3	Corn	V5	Min. till			Sparse LEPVI, TAROF, ERICA	Weeds burned down
4	Soybean	VC	No till			Very low	MATMT, TAROF, TRFRE in ditch
5	Soybean	V1	No till	Pioneer	Vacuum	Heavy STEME	Weeds burn-down
6	Corn	V6	Min. till	Agrigold	Vacuum	Few dead weeds	Early burn-down
7	Corn	V7	Min. till	Pioneer	Vacuum	Clean field	
8	Corn	V7	Min. till	Pioneer	Vacuum	Some dying STEME, LAMAM	Post sprayed field
9	Corn	V6	Min. till	Agrigold	Vacuum	TAROF, CHEAL on field edge	Some IPOHE, HIBTR emerged before post spray
10	Soybean	VC	No till			STEME, LAMAN,	Weeds burned down,

						SONAR	TAROF, TRFRE in ditch
11	Corn	V6	Min. till			Many STEME, some AMBTR on edges	Post sprayed field
12	Corn	V6	Min. till			STEME, LAMAN, LEPVI	AMBTR edges, post sprayed
13	None		Fall tilled			CHEAL, STEME	Bromegrass and wheat volunteering
14	Soybean	V1	No till			Sparse weeds	Weeds burned down, small field
15	Soybean	V1	No till	Agrigold	Vacuum	Sparse weeds	Weeds burned down
16	Corn	V6	Min. till			Sparse STEME	Burn-down
17	Soybean	VC	No till			CHEAL, STEME, LEPVI, CAPBP	Burn-down
18	Soybean	V1	No till			Winter annuals	Burn-down, small field
19	Soybean	VC	No till	Pioneer	Vacuum	Nearly clean	
20	Grass	Tillered	Hay field			Few weeds	
21	Corn	V6	Min. till	Pioneer	Vacuum	Sparse STEME, THLAR, CAPBP	Post sprayed
22	Corn	V7	Min. till	Pioneer	Vacuum	Patchy STEME, THLAR	Burned after flower
23	Sweet corn	V0, V3, V5	Tilled			Clean	Multiple sweet corn plantings
24	Orchard					Some veggies in front of orchard	Tilled area with cabbage, onions
25	Clover	Flowering					
26	Orchard		Orchard				
27	Soybean	V1	No till	Agrigold	Vacuum	Sparse weeds on edges	
28	Soybean	Emerged	No till			Clean	Small field
29	Corn	V5	Min. till			TAROF edges	Knifed in N, burn-down weeds
30	Soybean	VC	No till	Agrigold	Vacuum	Mixed winter annuals	Drilled
31	Soybean	V1	No till			Sparse winter annuals	
32	Soybean	V1	No till			Sparse STEME	Burn-down
33	Grass	Tillering				Sparse	Small field
34	Soybean	VC	No till			Clean	
35	Soybean	V1	No till			Clean	
36	Corn	V6	No till	Agrigold	Vacuum	Sparse STEME	
37	Soybean	V1	No till			Clean	
38	Corn	V6	Tilled			Volunteer wheat	Maybe cover crop
39	Soybean	V1	No till			Clean	Grassy ditch
40	Corn	V6	Tilled			Volunteer wheat	Maybe cover crop, knifed in N

41	Soybean	V2	No till			STEME, TAROF, LEPVI, VERAR, LAMAM	Burned off weeds, fairly weedy
42	Soybean	V1	No till	Pioneer	Vacuum	Sparse senescing STEME	
43	Corn	V6	Min. till			Sparse TAROF, SONAR	Recent burn-off
44	Soybean	V2	No till			STEME, VERAR, LEPVI, HPPVU	Weedy
45	Soybean	V1	No till			Sparse winter annuals	Burn-down
46	Corn	V5	No till	Pioneer	Vacuum	Sparse winter annuals on edges	Burn-down
47	Corn	V6	No till			Clean	Knifed in N
48	Corn	V6	Min. till	Agrigold	Vacuum	Nearly clean	
49	Corn	V4	No till			CAPBP	Winter annuals burned off
50	Corn	V6	No till	Agrigold	Vacuum	Patchy CAPBP	
51	Corn	V4	No till			Some CAPBP	
52	Corn	V7	Min. till			Sparse senescing STEME, CAPBP	
53	Soybean	V1	No till			clean	
54	Soybean	V1	No till	Pioneer	Vacuum	Nearly clean, VERAR, CAPBP	
55	Corn	V6	Min. till	Agrigold	Vacuum	Very sparse winter annuals	ALS applied

Weed key: TAROF = dandelion
CAPBP = Shepard's purse
PLALA = buckhorn Plantain
ERICA = marestail
AMBTR = giant ragweed
STEME = common chickweed
VERAR = corn speedwell
LEPVI = Virginia pepperweed
TRFRE = white clover
MEUOF = yellow sweetclover
LAMAM = henbit
HIBTR = Venice mallow
IPOHE = ivyleaf moringglory
SONAR = perennial field sowthistle
MATMT = pineapple weed
THLAR = field pennycress